

Supplemental Material

Peroxisome Proliferator-Activated Receptor γ is a Target for Halogenated Analogues of Bisphenol-A.

Anne Riu¹, Marina Grimaldi^{2,3,4,5}, Albane le Maire^{6,7}, Gilbert Bey⁸, Kevin Phillips⁹, Abelhay Boulahtouf^{2,3,4,5}, Elisabeth Perdu¹, Daniel Zalko¹, William Bourguet^{6,7} and Patrick Balaguer^{2,3,4,5*}

¹INRA, UMR 1089 Xénobiotiques, 31027 Toulouse Cedex 3, France ; ²IRCM, Institut de Recherche en Cancérologie de Montpellier, Montpellier, F-34298, France; ³INSERM, U896, Montpellier, F-34298, France ; ⁴Université Montpellier 1, Montpellier, F-34298, France;
⁵CRLC Val d'Aurelle Paul Lamarque, Montpellier, F-34298, France ; ⁶INSERM U1054, Centre de Biochimie Structurale, Montpellier, France ; ⁷CNRS UMR5048, Universités Montpellier 1 & 2, Montpellier, France ; ⁸NovAliX, 67400 Illkirch, France ; ⁹Methodist Hospital Research Institute, Houston, TX 77030

Table of Contents

Supplemental Table 1.

Data collection and refinement statistics p. 3

Supplemental Figure 1.

Chemical structures of MEHP, PFOA and PFOS. p. 4

Supplemental Figure 2.

TBBPA and TCBPA in the PPAR γ ligand-binding pocket. p. 5

Supplemental Figure 3.

Differences between human and zebrafish PPAR γ ligand-binding pockets. p. 6

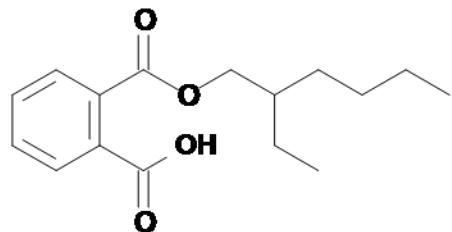
Supplemental Figure 4.

Comparison of the binding pockets in PPAR γ /ligand complexes. p. 7

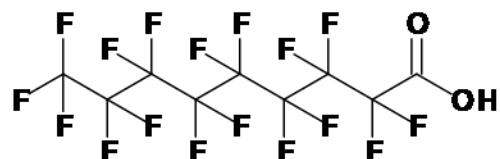
Supplemental Table 1. Data collection and refinement statistics

Complex	TBBPA	TCBPA
PDB code	3OSW	3OSI
Space group	<i>C</i> 2	<i>C</i> 2
Cell dimensions a, b, c (Å)	93.09, 61.69, 118.46	92.93, 61.74, 118.64
β (deg)	102.75	102.79
Resolution range (Å)	48.97-2.55 (2.69-2.55)*	44.70-2.70 (2.85-2.70)*
No. of reflections	20873	18014
R _{sym}	0.068 (0.435)*	0.070 (0.345)*
I/SI	9.5 (2.1)*	9.3 (2.7)*
Completeness, %	96.6 (92.7)*	98.9 (99.1) *
Redundancy	2.9 (2.9)*	2.3 (2.3)*
Refinement		
Resolution range (Å)	45.40-2.55	44.70-2.70
R (%) /R _{free} (%)	23.4 /29.6	23.2/29.6
Number of atoms	4206	4145
No. protein atoms	4074	4028
No. ligand atoms	21	21
No. water molecules	106	91
Average <i>B</i> -factor (Å ²)		
Protein <i>B</i> -factor (Å ²)	47.63	48.62
Ligand <i>B</i> -factor (Å ²)	57.75	55.27
Water <i>B</i> -factor (Å ²)	43.72	38.98
Rmsd from ideality		
Bond lengths (Å)	0.008	0.008
Angles (°)	1.1	1.2
Ramachandran plot (%)		
Favored region	93.0	93.4
Additionally allowed regions	6.6	6.4
Generously allowed regions	0.4	0.2
Disallowed regions	0.0	0.0

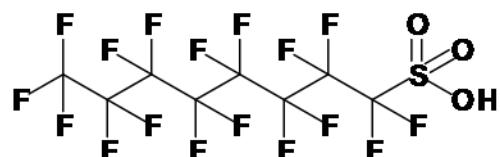
*Values in parentheses are for highest resolution shell.



Mono-2ethylhexyl phthalate (MEHP)

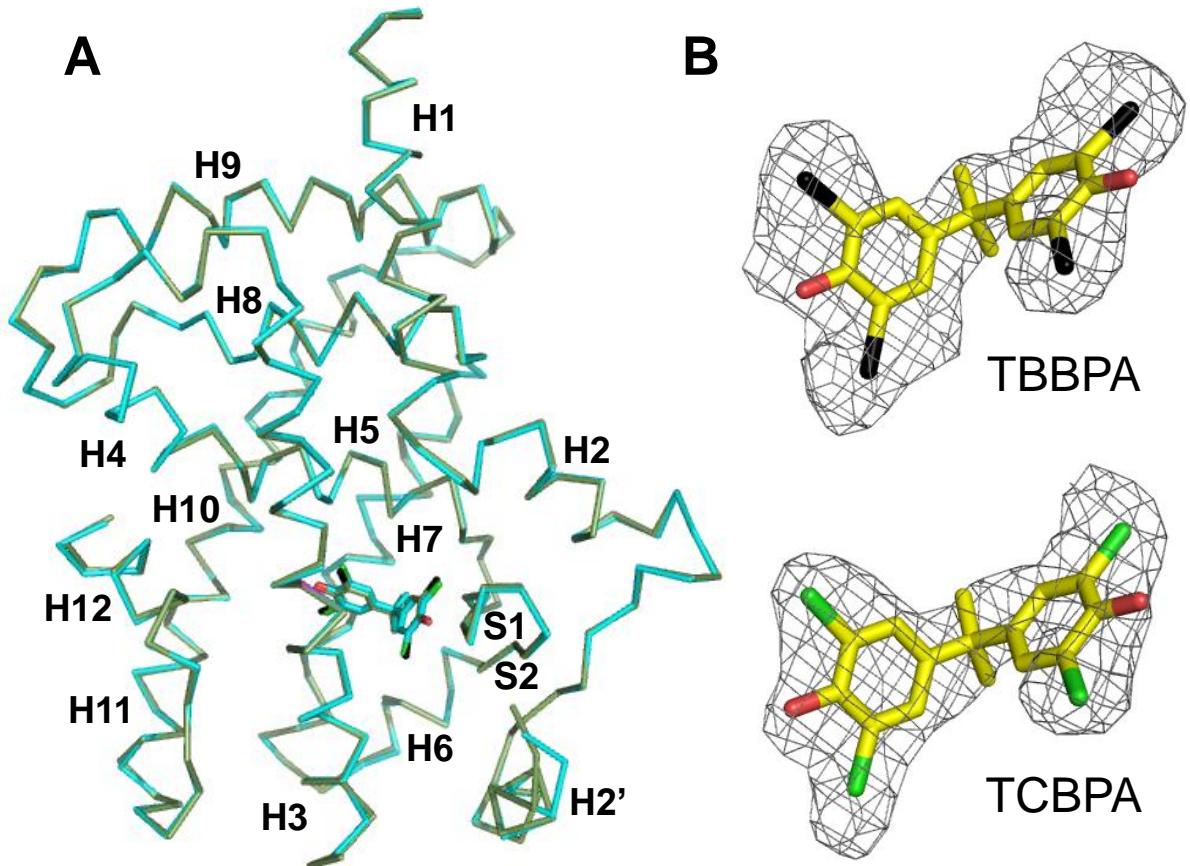


Perfluorooctanoic acid (PFOA)



Perfluorooctanesulfonic acid (PFOS)

Supplemental Figure 1. Chemical structures of MEHP, PFOA and PFOS.

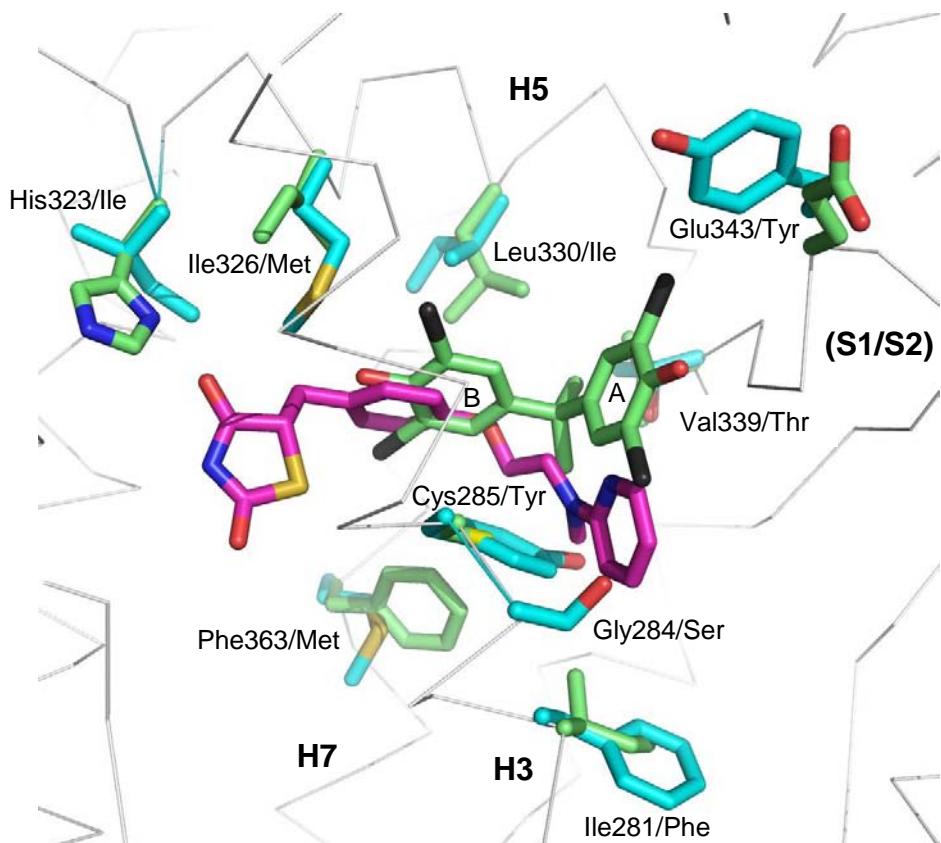


Supplemental Figure 2. TBBPA and TCBPA in the PPAR γ ligand-binding pocket. (A) Carbon trace of superimposed PPAR γ monomers bound to TBBPA (green) and TCBPA (cyan). (B) TBBPA and TCBPA in their respective Fo-Fc omit maps contoured at 2.5σ .

A

275	285	295	305	315	325	335	345	355
**	***	*	*	*	*	*	***	**

hPPAR KEVAIR**I****FQGCQFRSVEA**VQEITEYAKSIPGVNL~~D~~N~~D~~QVTLLKYGV**H**E**I****I****YTMLASLMNKDGVLISEGQQGFM**TREFL**K**SLRKPFGD**FME**
 mPPAR KEVAIR**I****FQGCQFRSVEA**VQEITEYAKNIPGFIN~~D~~LNDQVTLLKYGV**H**E**I****I****YTMLASLMNKDGVLISEGQQGFM**TREFL**K**NLRKPFGD**FME**
 zPPAR HEVELR**FFH****SYQSRSAAE**ISEVTEFAKSIPGFIN~~D~~LNDQVTLLKYGV**I****EVM****I****M****I****SPLMNKDGT****LISY****GQIFM**TREFL**K**SLRKPFC**E****M****E**

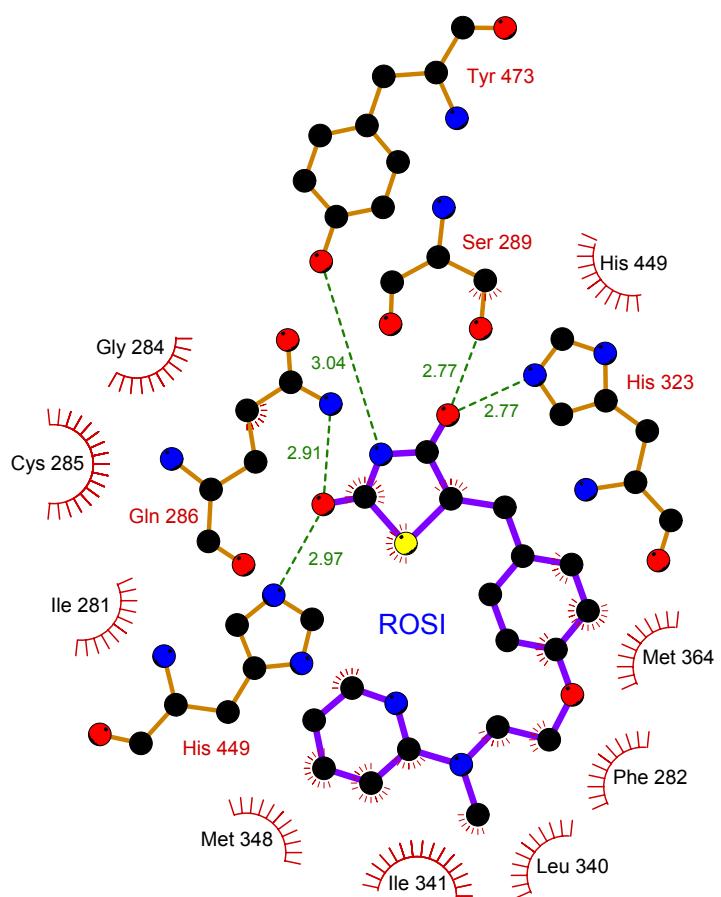
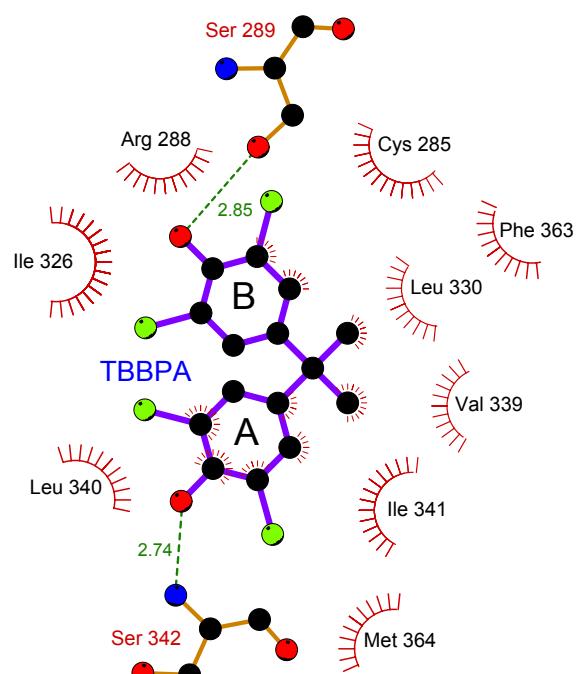
B

Supplemental Figure 3. Differences between human and zebrafish PPAR γ ligand-binding pockets. (A) Sequence alignment of human, mouse and zebrafish PPAR γ ligand binding pocket residues. Asterisks denote residues in contact with TBBPA and/or rosiglitazone (PDB code 2PRG). Interacting residues that differ between sequences are highlighted in red. (B) TBBPA (carbon atoms colored in green) and rosiglitazone (magenta, PDB code 2PRG) as they are positioned in the human PPAR γ . Residues that differ in the ligand binding pocket of human and zebrafish PPAR γ are displayed as green and cyan sticks, respectively.

A

Ligand	Contacts (total) ($< 4.2 \text{ \AA}$)	Hydrogen bonds	van der Waals	IC50
Rosiglitazone	94	5	89	12.0 nM
TBBPA	81	2	79	0.7 μM
TCBPA	77	2	75	6.0 μM

B



●—● Ligand bond

●—● Non-ligand bond

●—●—● Hydrogen bond and its length

Non-ligand residues involved in hydrophobic contacts ● Corresponding atoms involved in hydrophobic contact(s)

Supplemental Figure 4. Comparison of the binding pockets in PPAR γ /ligand complexes. (A) Number of contacts (total, hydrogen bonds, van der Waals) and IC50 characterizing the complexes between PPAR γ and rosiglitazone, TBBPA and TCBPA. (B) Schematic drawing showing the interactions between PPAR γ and the ligands in the TBBPA (left) and rosiglitazone (right) complexes.